

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Higuchi

Serial No.: 08/968,208

Filed: November 12, 1997

For: **HOMOGENEOUS METHODS FOR NUCLEIC
ACID AMPLIFICATION AND DETECTION**

) Group Art Unit: 1743

) Examiner: J. Snay

For hand delivery to Examiner
J. Snay in Group 1743 at 703-308-
4032 on or after July 20, 2001

Declaration of Timothy M. Woudenberg
Under 37 CFR 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Timothy M Woudenberg, declare and affirm as follows:

1. I am currently an employee of Applied Biosystems, a business group of PE Corporation (NY), where I am Director of Scientific Operations in Foster City, California. PE Corporation (NY) has an ownership interest in the subject matter of the present application.

2. I received a Bachelor of Science Degree in Chemistry from Purdue University in 1980. I received a Ph.D. Degree in Chemistry from Tufts University in 1988, where I specialized in fluorescence spectroscopy. From 1987 to 1993, I was employed by The Perkin-Elmer Corporation ("Perkin-Elmer") in Connecticut as an Engineer II, where I worked on projects relating to thermal cyclers and other instrumentation for use with PCR (polymerase chain reaction). I was promoted to Senior Engineer in 1989. In 1993, I transferred to Applied

Biosystems (which had just been acquired by Perkin-Elmer in late 1992), where, among other things, I have worked on the development of instrumentation for PCR detection of target nucleic acids for use in basic scientific research, forensics, diagnostics, and the like.

3. I have authored or co-authored at least nine scientific journal articles relating to fluorescence spectroscopy, thermal cycling equipment, analytical devices, and/or PCR. I am an inventor on at least 14 issued United States patents, as well as other pending patent applications and foreign counterparts thereof.

4. I have reviewed the above-identified patent application, the pending claims, the Office actions mailed May 23, 2000, November 29, 2000, and February 27, 2001, and the references cited therein. From reading these Office actions, I understand that the claims stand rejected for allegedly being obvious over Haff et al. ("Measurement of PCR Amplification by Fluorescence" in Amplifications 1:8-10, 1989) in view of Mackay (EP 266881 A2). For the following reasons, I respectfully disagree.

5. The Haff article describes an assay in which a series of identical PCR reaction mixtures (100 μ L each, according to the paragraph under "Principle" on page 8) were run in parallel for up to 25 PCR thermal cycles. After a selected number of cycles (4, 8, 12, 14, 16, 18, 20 and 25 -- see Figure 1), a 25 μ L sample (aliquot) was withdrawn manually from a successive reaction mixture by pipet. To each aliquot was added 200 μ L of dye solution (Hoechst 33258, 2 μ g/mL after 1:500 dilution of 1 mg/mL stock solution) and about 1.775 μ L of TE buffer to make a final assay volume of 2.0 mL (see third paragraph under "Method" on page 9). The obtained fluorescence values are plotted in Figure 1.

6. One thing that is apparent from the Haff article is that the fluorescent detection dye was not present in the PCR reaction mixture during amplification. Rather, the dye was added to an aliquot of a given sample only after a selected number of thermal cycles had been completed.

7. The approach described in the Haff article suffers from several shortcomings. For example, each reaction mixture must be opened to allow removal of a sample aliquot for assay. The presently claimed apparatus allows monitoring of a PCR reaction without opening the sample. Also, multiple reaction mixtures (rather than one) had to be run in parallel for the same DNA sample, thereby increasing the overall assay time and number of pipetting steps for the user, and introducing potential error in the results due to differences in PCR reaction kinetics that

might occur in the different reaction mixtures, e.g., due to pipetting errors or variations in heating and cooling. The present invention avoids such risks by allowing data to be collected from a single reaction mixture.

8. Although the Haff article mentions the possibility of automating his method on page 9 under "Instrumentation", the cited device (a Perkin-Elmer Model LS-2B spectrophotometer with an autosampler) would only have automated the transfer of each assay mixture (formed by mixing an aliquot of a PCR reaction mixture with dye solution and TE buffer as discussed in paragraph 5 above) from an autosampling tray into and out of the fluorescence cuvette for fluorescence measurement. The cited autosampler had no capacity to automate the collection of aliquots from the PCR reactions, nor mixing of those aliquots with dye solution, so these steps would still need to be done by the user.

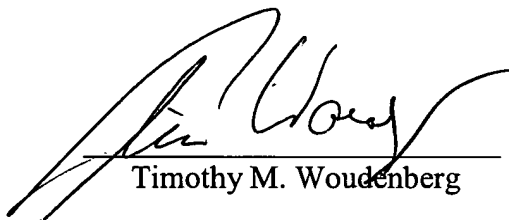
9. The Mackay reference shows an optical assay system of the type used for analysis of immunoassay samples, but modified to be able to detect two different markers in the same sample mixture. There is no mention of nucleic acid amplification, nor employment of a thermal cycler.

10. Until the first parent of the present application was filed (May 2, 1991), it was unknown in the field of PCR to monitor the course of PCR amplification as a function of the number of thermal cycles without withdrawing an aliquot for analysis outside of the original reaction mixture. One reason for this is that there was a general belief by practitioners of PCR that duplex nucleic acid specific dyes like ethidium bromide would interfere with assay performance, e.g., by inhibiting the polymerase used to perform primer extension. This is reflected, for example, in the teachings in Kornberg's DNA Replication text cited in the present application on page 4, line 36 through page 5, line 10 (A. Kornberg, DNA Replication, W.H. Freeman and Co., San Francisco, 1980, pages 427-441), which were still present in his 1992 edition (A. Kornberg, DNA Replication, Second Edition, W.H. Freeman and Co., New York, 1992, pages 451-455), copies of which are attached hereto. In my opinion, the present inventor's discovery that such a dye could be included in a PCR reaction mixture without compromising the results was an unexpected result. Furthermore, the combination of an optical system and thermal cycler for real time monitoring of PCR amplification had not been suggested in the art.

11. In my view, the present invention provided a significant advance in nucleic acid detection which has implemented broadly in the biochemical sciences.

12. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

7/18/01
Date


Timothy M. Woudenberg

Attachments:

- A. Kornberg, DNA Replication, W.H. Freeman and Co., San Francisco, 1980, pp427-441
A. Kornberg, DNA Replication, 2nd Ed., W.H. Freeman and Co., New York, 1992, pp451-455